Research Article

Identification of Multi-epitopes as an effective vaccine against Chikungunya virus: An Immunoinformatics Study

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Abstract : Chikungunya, a virus that is transmitted to humans by the bite of an infected mosquito. Chikungunya is caused by the Chikungunya virus (CHIKV), an RNA virus belonging to the alphavirus genus and Togaviridae family. The term Chikungunya comes from the Kimakonda language and means "to become contorted." The most common symptoms include fever, headache, and joint pain that persist. Africa, Southeast Asia, India, and Brazil are endemic regions for Chikungunya fever. The primary objective of this work is to identify an effective epitope based subunit vaccine against the virus through computational analysis and a reverse vaccinology approach. T cell epitopes were predicted and evaluated for antigenicity and population coverge based on the CHIKV structural proteins. In this, the epitope PRNVELGDRKGK exhibited the highest antigenicity score of 2.4092, indicating its strong potential as an immunogenic candidate. Also, the population coverage analysis revealed its global effectiveness with 71.70% coverage. Further, protein-peptide docking of the modelled peptide with the CHIKV envelope glycoprotein identified the most stable molecular complex based on a lowest binding energy of -556.0 kcal/mol, with key interactions were visualized. This immunoinformatics investigation demonstrates that the anticipated epitopes are capable of inducing adequate antibodies against CHIKV and suggests that their epitopes can be exploited in the development of the novel effective and successful CHIKV vaccine.

Keywords: vaccine; chikungunya; epitopes; antigenicity; population coverage; immunoinformatics.

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Introduction

Chikungunya virus (CHIKV) is a type of alphavirus spread mainly by female Aedes mosquitoes, particularly *Aedes albopictus* and *Aedes aegypti*, which thrive in urban areas and are the primary carriers of the virus [1]. As an emerging infectious disease, CHIKV presents serious global health concerns due to its widespread socioeconomic impact and high rates of illness, making treatment a challenging task [2]. The virus was first identified in 1952 on the Makonde Plateau in south-eastern Tanzania. The name "Chikungunya" comes from the Kimakonde word "kungunyala," which means "to become contorted" or "to walk bent over," describing the severe joint pain experienced by those infected [3].

Interestingly, the virus can also be transmitted between mosquitoes through mating, with infected male *Aedes aegypti* passing the virus to females [4]. People who are older or have pre-existing conditions like obesity,

autoimmune disorders, heart failure, and diabetes face a greater risk of severe complications [5]. Unlike some other viruses, CHIKV does not spread directly from person to person, except in cases where an infected mother transmits it to her child at birth. Instead, mosquitoes pick up the virus when they bite infected humans or non-human primates and then pass it on to new hosts [6].

CHIKV can lead to serious health problems, including neurological complications and, in rare cases, death [7]. After being bitten by an infected mosquito, symptoms usually appear within 3 to 7 days. The illness often begins with a fever that can last up to two weeks and may include other symptoms, though some people experience no symptoms at all [8]. Researchers have identified three major CHIKV lineages, each associated with specific geographic regions: the West African lineage, the East-Central-South African (ECSA) lineage, and the Asian lineage [9]. The Indian Ocean lineage is a notable sub-group of the ECSA lineage [10].

In the early stages, CHIKV infection causes fever, headaches, chills, light sensitivity, skin rashes, and intense joint pain [11]. Chronic symptoms, like lingering or recurring joint pain and arthritis, affect nearly 40% of patients, significantly reducing their quality of life. However, scientists are still trying to understand why these long-term effects occur [12]. In 2004, CHIKV reappeared on the Kenyan coast and soon spread across South Asia and La Réunion, driven by genetic changes in its E1 (A226V) and E2 glycoproteins. These mutations helped the virus adapt from *Aedes aegypti* to *Aedes albopictus*, enabling it to spread to new climates and regions [13].

Over the past decade, CHIKV has moved beyond tropical Africa and nearby islands to the Americas. The first local transmission in the Caribbean was reported in late 2013, and by October 2020, the virus had reached 45 countries across all continents except Antarctica [14]. The illness progresses in three phases: acute, sub-acute, and chronic [15]. One of the most severe outbreaks occurred in La Réunion in 2005–2006, infecting nearly 260,000 people—more than one-third of the island's population—with 40,000 new cases per week and 284 deaths [16]. Between 2013 and 2019, the Pan American Health Organization recorded 631 CHIKV-related deaths in South America, but the true death toll is likely higher [17].

In La Réunion, researchers confirmed that CHIKV can be transmitted from mother to fetus, with viral genomes found in amniotic fluid, the placenta, and fetal brain tissue. Affected mothers tested positive for the virus via RT-PCR two weeks before fetal loss, showing that the virus remained in the placenta and amniotic fluid after the baby had passed [18]. As of July 1, 2023, there is still no approved vaccine or antiviral treatment for CHIKV. Current management focuses on relieving symptoms, such as using paracetamol or acetaminophen for fever and pain relief [19]. However, studies suggest that CHIKV-neutralizing antibodies (NAbs), whether from natural infection or vaccination, can provide protection in mice, making them a key target for vaccine development [20].

The CHIKV genome is about 12 kilobases long and codes for five

structural proteins (E1, E2, E3, capsid, and 6K) and four non-structural proteins (nsP1–nsP4). The E1 and E2 glycoproteins are critical for viral entry: E2 helps the virus attach to host cells, while E1 enables membrane fusion [21]. These proteins are essential for the virus's life cycle and are prime targets for vaccine research. The immune system recognizes specific parts of these viral proteins, called epitopes, which trigger an immune response. Toll-like receptors also play a key role in detecting the virus and activating immune defenses [22].

In this study, we aimed to identify a strong epitope candidate for a CHIKV vaccine. Using computational tools, we analyzed various databases to predict potential epitopes. We identified T-cell epitopes, including those recognized by MHC class I and MHC class II molecules, and designed a multi-epitope vaccine construct. This construct was further assessed for its antigenicity, population coverage, and ability to bind to the viral envelope protein, offering valuable insights into its potential as a vaccine candidate.

Materials and Methods

Protein Sequence Retrieval

The structural polyprotein sequence of Chikungunya virus (CHIKV), specifically from the Nagpur strain, was retrieved from the UniProt database (https://www.uniprot.org) (accession number: Q5WQY5). This sequence, consisting of 1,248 amino acids, was selected for its potential relevance in vaccine design and immune response predictions.

T-Cell Epitope Prediction

In the human population, Major Histocompatibility Complex I (MHC-I) molecules are encoded by human leukocyte antigen (HLA) alleles and are essential for presenting antigens to T cells [23]. In this study, the retrieved protein sequence was submitted to the IEDB server's MHC-I T-cell epitope prediction tool, where it was processed to identify peptides capable of binding to the HLA-A*01:03 allele [24]. This analysis identified potential T-cell epitopes, each consisting of 12 amino acids, based on their binding affinities and ability to interact effectively with the selected MHC-I molecule.

Antigenicity Prediction

The antigenicity analysis was conducted using the VaxiJen v2.0 online server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) [25] with a threshold of 0.6. This tool predicts epitopes based on their physicochemical properties, classifying peptides with scores higher than the threshold as potential epitopes [26]. The antigenicity scores assigned to the predicted epitopes are presented in Supplementary Table 1.

Population Coverage

The population coverage analysis for the predicted epitopes was performed using the IEDB population coverage tool to assess global populations [27]. The analysis estimated the distribution of MHC-I epitopes based on their binding to the HLA allele, providing insights into the potential

effectiveness of these epitopes in diverse populations.

Prediction of the 3D Structure of the Selected Epitope

Based on antigenicity and population coverage analysis, the identified epitope was modeled using PepFold3, a tool for de novo peptide structure prediction, to predict its three-dimensional structure [28]. Furthermore, the modeled epitope was analyzed for its interaction with the protein to assess its potential binding and immune response.

Molecular Docking

The three-dimensional structure of the HLA-A*01:03 allele (PDB ID: 6JO8) was obtained from the Protein Data Bank (PDB) (https://www.rcsb.org) and optimized for docking studies. Protein-peptide docking was conducted using ClusPro 2.0, a web-based server designed for generating reliable docking models [29]. Out of over 30 predicted models, those with the best center energy score and the lowest energy score were selected for detailed analysis to ensure accuracy and stability.

Interaction Analysis

The interaction between the epitope and the protein was analyzed using BIOVIA Discovery Studio Visualizer 2024 to identify the amino acid residues involved in binding [30]. These residues were examined for their contribution to binding stability and specificity, providing detailed insights into molecular interactions. This analysis is essential for understanding the epitope's role in the immune response and optimizing its design for epitope-based vaccine development.

Results and Discussion

Malaria is a mosquito-borne disease that has a severe impact on human health. Similarly, CHIKV is a mosquito-borne disease whose severity leads to significant economic consequences, particularly in poorer nations, and affects human life. In extreme cases, CHIKV infections can be fatal due to mutations in the viral genome. Therefore, preventing this threatening virus is essential. The primary focus of this research is on the concept of "Epitope-Based Peptide Vaccine Design" , a logical approach since conventional vaccine development is slow and antigen selection is largely random. The genome of the Chikungunya virus consists of a 12 kb single-stranded positive-sense RNA, encoding non-structural proteins essential for replication and structural proteins necessary for viral assembly, host attachment, and immune response modulation. The E2 and E1 proteins play crucial roles in binding to host receptors and influencing immune responses, featuring conserved epitopes within domain A of E2. Additionally, *in silico* techniques facilitate vaccine development in a cost-effective manner [31].

Epitope-based vaccines offer advantages over conventional vaccines by addressing safety concerns and enhancing immunogenicity through precise antigen presentation via MHC molecules. The use of HLA supertypes improves

population coverage and reduces antigen escape, ensuring effective T-cell activation and immune response [32]. The Chikungunya virus (CHIKV), a re-emerging arbovirus transmitted by mosquitoes, represents a major global public health challenge [33]. Currently, there are no licensed vaccines or effective antiviral treatments for CHIKV. Since management is limited to supportive care, there is an urgent need for the development of prevention and treatment strategies [34]. The primary structure of the target sequence was retrieved from the UniProt database (UniProt ID: Q5WQY5, strain Nagpur). The amino acid sequence of the structural polyprotein was obtained in FASTA format for predicting T-cell epitopes. MHC-I binding epitopes specific to the human leukocyte antigen (HLA) allele HLA-A*01:03 were predicted using the IEDB server, resulting in a total of 1,238 epitopes. The best epitopes were selected based on their antigenicity scores, ensuring their potential for effective immune response induction.

Antigenicity analysis of the peptides was performed using the VaxiJen v2.0 tool with a threshold value of 0.4. Among the 1,238 epitopes, the epitope PRNVELGDRKGK exhibited the highest antigenicity score of 2.4092, indicating its potential as an immunogenic candidate. These findings provide valuable insights into the antigenic properties of the analyzed peptides and their epitopes, emphasizing their relevance in vaccine design and immunotherapy development.

Furthermore, the immunogenic response of epitope-based vaccines can vary significantly due to the genetic diversity of human HLA alleles across different ethnic groups and regions. To address this variation, the overall population coverage for the selected epitope was assessed using the IEDB Population Coverage analysis tool. Notably, the epitope PRNVELGDRKGK demonstrated a global population coverage of 71.70%, highlighting its potential effectiveness on a worldwide scale (Fig. 1).

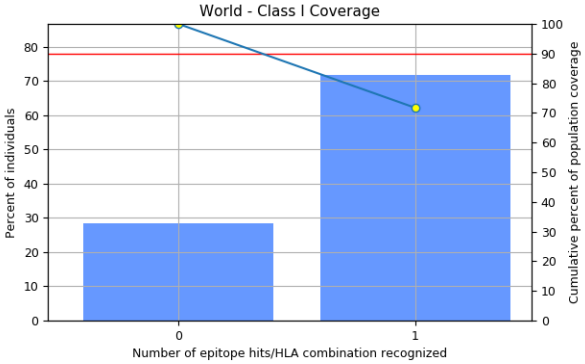

Figure 1. Population coverage analysis of the epitope PRNVELGDRKGK worldwide. The chart represents the percentage of individuals recognizing the epitope based on HLA Class I coverage.

These results underscore the potential of the epitope-based vaccine to generate a robust immune response and offer effective protection against

CHIKV across diverse populations globally. The PEP-FOLD3 server was employed to determine the optimal model configurations and to simulate the three-dimensional structures of the chosen peptides. The peptide (PRNVELGDRKGK) sequence was submitted for modelling. A simulation setting of 100 was applied to rank the generated models. The most appropriate model was selected (Fig. 2) and subsequently used for molecular docking with its corresponding alleles, including the HLA-A*01:03 allele (PDB ID: 6JO8), for MHC-I epitope analysis.

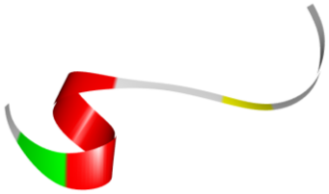

Figure 2. PEP-FOLD3 predicted 3D structure of the epitope

The complex structure of the CHIKV envelope glycoprotein bound to human MXRA8 was obtained from the Protein Data Bank (PDB), and protein-peptide docking for the modeled peptide and the glycoprotein was performed using ClusPro 2.0. The docking results generated 13 models, with the most stable molecular complex identified based on the lowest binding energy of -556.0 kcal/mol. More negative binding energy values correlate with the formation of stable molecular complexes, which are crucial for the activation of biological responses. This stability plays a significant role in glycoprotein-peptide binding formation, potentially triggering processes such as receptor-mediated endocytosis during CHIKV viral entry. The docked complex was visualized using BIOVIA Discovery Studio.

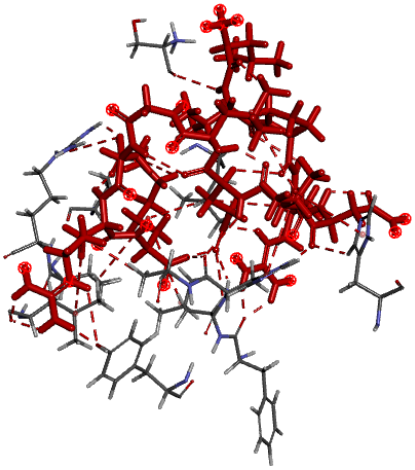

Figure 3. Docking complex of the modeled peptide and the envelope glycoprotein of CHIKV

Among all the refined 3D structures, model 2 was selected for its superior quality, and the structure was visualized using Discovery Studio 2020 (Fig. 3). During peptide-protein binding, various interactions came into play, including hydrogen bonds, hydrophobic interactions, and electrostatic interactions (Fig.

4).

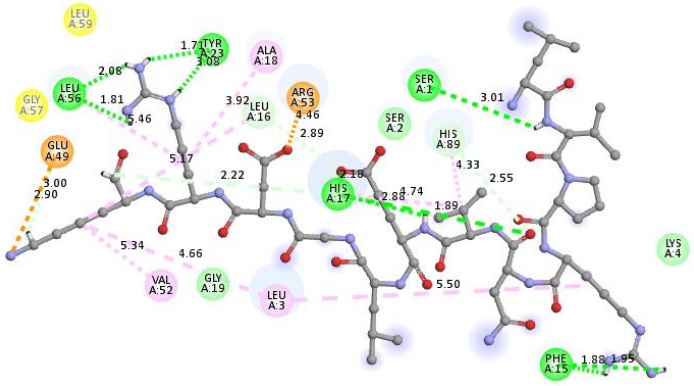

Figure 4. 2D diagram of the docking complex highlighting the key interacting amino acid residues involved in the binding interface between the modelled peptide and the envelope glycoprotein of Chikungunya virus (CHIKV).

Specifically, the amino acid residues His17, Ser1, Phe15, Tyr23, and Leu56 promoted hydrogen bond interactions, while Arg53 and Glu49 facilitated electrostatic interactions. Furthermore, the residues Ala18, Val52, and Leu3 were involved in hydrophobic interactions. These findings provide key insights into the stability of the peptide-glycoprotein complex and its relevance in developing effective therapeutic strategies targeting CHIKV.

Conclusion

This work focused on epitope-based immunoinformatics design of vaccine against CHIKV. Even though it is very difficult to commence research on CHIKV as it demands bio-safety level 4 (BSL-4) and the high chances to become endemic and the lack of effective therapies posts a significant threat to the human community. To this end, the predicted epitopes in this study may play a vital and informative role in the production of vaccines against CHIKV. Also, the common antigenic peptides revealed in this study may also play a vital role in the design of synthetic peptide vaccines. We anticipate that our present findings will assist pharmacologists and scientific research community to choose the potential epitopes which can be helpful for future vaccine designing against CHIKV.

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